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PATENT APPLICATION
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#23

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Application No.: 08/700,737 Group Art Unit: 1644

Filed: August 15, 1996 Examiner: P. Gambel

For: HUMANIZED IMMUNOGLOBULIN REACTIVE WITH α4β7 INTEGRIN

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Assistant Commissioner for Patents, Washington, D.C. 20231 on 01/18/00 (Date) Lisa Jensen (Signature)

Lisa Jensen
Typed or printed name of person signing certificate

DECLARATION OF STEVEN B. LANDAU, M.D. UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Steven B. Landau, M.D., of 44 Tanglewood Road, Wellesley, Massachusetts, hereby declare and state that:

1. I received a Bachelor of Arts degree in Chemistry in 1982 from Bowdoin College, Brunswick, Maine, and a degree in medicine in 1986 from Case Western Reserve University, Cleveland, Ohio.

2. I have been employed in the pharmaceutical industry since 1996. In particular, I have been employed by LeukoSite, Inc. since 1998 to present, serving as Medical Director with responsibility for clinical projects in the areas of inflammatory bowel disease, stroke and rheumatoid arthritis. From 1997 to 1998, I was employed as Medical Director by Hurley Consulting, Chatham, New Jersey, and from 1996 to 1997, I was employed as Medical Director by OraVax Inc., Cambridge, Massachusetts.

3. I held a hospital appointment from 1993 to 1996 as Attending Physician at the Fletcher Allen Health Care/Medical Center Hospital of Vermont.

4. Since the filing of the subject application, further clinical studies of the administration of anti-$\alpha 4\beta 7$ antibodies of the present invention have been performed on behalf of the Assignee of the subject application, LeukoSite, Inc. The work described herein relates to pharmacokinetic and pharmacodynamic studies of the administration of an anti-$\alpha 4\beta 7$ antibody of the present invention. The study was performed at Medeval Limited in Manchester England, a Phase I Clinical Study Unit, under the direction of Principal Investigator Dr. Paul Rolan, with a protocol modified, approved and sponsored by LeukoSite, Inc.

Drug Substance and Formulation

LDP-02 is a humanized monoclonal antibody directed against the $\alpha 4\beta 7$ integrin. It was humanized by grafting the complementarity determining regions (CDRs) from the murine monoclonal antibody, ACT-1, onto human variable regions as described in Examples 2-4 of the subject application

The active ingredient (LDP-02) was chromatographically purified from medium of fermentor-grown cells and supplied as an aqueous solution of purified antibody. LDP-02 was supplied in injection vials as a purified preparation in 20 mM isotonic sodium citrate buffer (pH 6.0) containing 5 mg of antibody in a volume of 1.0 mL. Each vial contained a nominal volume of 2 mL. The 20 mM isotonic sodium citrate buffer contained: 0.48 mg citric acid, 5.21 mg trisodium citrate, and 7.31 mg NaCl, in 1 ml sterile water for injection. The concentrated solution was kept frozen at ≤-40°C. For intravenous administration, the drug was diluted in 0.9% sodium chloride solution. The intravenous infusion line contained an in-line 0.22 μm low protein-binding

(e.g. PES; polyethersulfone) filter. Low dosages given by subcutaneous injection were filtered through a 25 mm, 0.22 μm clinical grade filter.

Pharmacokinetics and Pharmacodynamics of LDP-02 in Healthy Subjects

This randomized, double-blind, placebo-controlled, ascending single-dose study was conducted by Medeval Limited, a Phase I Clinical Study Unit in Manchester, England under the direction of Principal Investigator, Dr. Paul Rolan.

Subjects meeting all inclusion/exclusion criteria were enrolled in the study sequentially by study group and, within each study group, were randomly assigned to receive LDP-02 or placebo (i.e., isotonic sodium citrate buffer). To minimize risk to subjects, safety and tolerability were reviewed at each dose level prior to escalating to the next dose level. The treatment groups and numbers of subjects are shown in Table 1.

Table 1. Treatment Groups and Number of Subjects

Group	Route of Administration*	LDP-02		Placebo	Total # enrolled
		# subjects	Dose	# subjects	
1	IV	3	0.15 mg/kg	1	4
	SC	3	0.15 mg/kg	1	4
2	IV	3	0.50 mg/kg	1	4
3	IV	3	1.5 mg/kg	1	4
4	IV	2	2.5 mg/kg	1	3
1-4	Total # subjects	14		5	19

*SC = subcutaneously; IV = intravenously

On study Day 1, LDP-02 or placebo was administered either SC into the thigh or via a 30 minute constant rate IV infusion. Safety assessments included physical examinations, vital signs, clinical laboratories (i.e., hematology, blood chemistries, and urinalysis), cytokine levels, 12-lead electrocardiograms (ECGs), and recording of adverse events. In addition, continuous cardiac monitoring was carried out pre-dose through 4 hours post-dose. Blood samples for determination

of the plasma profile of LDP-02 were obtained. Plasma cytokine levels were determined as were saturation and binding sites occupation of $\alpha 4\beta 7$ receptors and lymphocyte subsets. Study assessments were scheduled at various times through 36 days post- treatment.

Results

Subjects. A total of 19 healthy male subjects were entered. These subjects ranged from 19 to 46 years of age (mean = 29.4 years) and weighed from 62.6 to 104.5 kg (mean = 76.8 kg). Fifteen subjects (79%) were Caucasian, 3 (16%) were Black, and 1 (5%) was Asian. Eleven subjects received a single IV dose of LDP-02. Three received a single SC dose of LDP-02, and 5 subjects received placebo. All 19 subjects completed the trial.

Pharmacokinetic Data: Blood draws were originally scheduled through 36 days post-treatment. However, when it became known that LDP-02 was still detectable at Day 36, subjects who received LDP-02 returned for follow up beyond Day 36 for continued monitoring of LDP-02 concentrations, $\alpha 4\beta 7$ saturation, and memory cell populations to ensure these had returned to baseline levels or had fallen below the limits of quantitation.

Pharmacokinetic parameters after administration of single SC or IV doses of LDP-02 are summarized below (Table 2).

Table 2. Pharmacokinetic Parameters of LDP-02 in Healthy Subjects[1]

Pharmacokinetic Parameter	Dose and Route of Administration of LDP-02 (number of subjects)				
	0.15 mg/kg SC (n = 3)	0.15 mg/kg IV (n = 3)	0.5 mg/kg IV (n = 3)	1.5 mg/kg IV (n = 3)	2.5 mg/kg IV (n =2)
C_{max} (µg/mL)	1.112 (0.519)	7.648 (3.201)	15.760 (7.476)	118.813 (14.544)	101.749 (5.117)
t_{max} (days) (median & range)	6.01 (4.01 - 6.01)	0.13 (0.04 - 0.33)	0.5 (0.06 - 0.5)	1.13 (0.06 - 0.33)	0.05 (0.04 - 0.06)
$t_{1/2}$ (days)	4.33 (2.23)	4.39 (1.51)	4.02 (0.71)	14.9 (10.3)	17.1 (8.91)
AUC_i (µg•day/mL)	10.4 (4.40)	19.5 (5.00)	83.6 (18.3)	660 (229)	1651 (229)
λ_z (1/day)	0.1852 (0.0735)	0.1731 (0.06730	0.1763 (0.0344)	0.0994 (0.1145)	0 0469 (0.0244)
AUC (µg•day/mL)	11.4 (5.80)	20.3 (5.88)	85.1 (18.2)	755 (308)	1747 (95.8)
AUC Extrapolated %	5.9 (7.3)	3.4 (3.2)	1.8 (1.4)	9.5 (16.1)	5.7 (8.0)
CL* (mL/day/kg)	15.3 (6 26)	7.75 (1.93)	6.06 (1.32)	2.31 (1.19)	1.43 (0.08)
V_z* (mL/kg)	82.5 (6.88)	46.6 (10.1)	34.3 (2.84)	54.0 (51.4)	35.9 (20.3)

[1]All values are mean +/- SD unless otherwise indicated. The SD appears in parenthesis.

*Clearance and volume terms for the SC dose group are the apparent clearance (CL/F) and apparent volume (V_z/F).

The range of values obtained for the mean single dose IV pharmacokinetic parameters for the 4 dose groups (C_{max}, $t_{1/2}$ and AUC) suggest that non-linear pharmacokinetics were evident. The follow-up samples (i.e., those taken beyond Day 36), allowed some further characterization of the concentration-time profiles so that the amount of extrapolated AUC was small. The non-linearity in AUC and C_{max} could be linked to the fact that the assay of LDP-02 gave free LDP-02 concentrations and there were no free α4β7 sites detected following administration of the lowest dose (0.15 mg/kg). The non-compartmental pharmacokinetics of the lower doses of LDP-02 (0.15 and 0.5 mg/kg) were well characterized and non-linear pharmacokinetics became evident as the dose was increased up to 2 5 mg/kg.

Pharmacodynamics: Saturation of α4β7 integrin on lymphocytes: As measured by Fluoresence Activated Flow Cytometry (FACS) analysis, mean saturation of α4β7 integrin on lymphocytes over time (i.e., to Day 36) for each treatment are presented in the Figure.

As seen in the Figure, there was no detection of free α4β7 binding sites on lymphocytes for approximately two weeks following administration of LDP-02 as a single 0.15 mg/kg IV or SC dose. At the higher doses studied (0.50, 1.5, and 2.5 mg/kg), saturation of α4β7 appeared to persist for a month or longer following single IV doses. For the 2.5 mg/kg dose group saturation was found to persist up to Day 70 (data not graphed). FACS assessments cannot discriminate among saturations ≥85%; therefore, these initial doses achieved maximal detectable saturation of α4β7 on peripheral lymphocytes of at least 85%. Depending upon dose and the degree of saturation required for clinical benefit, doses of LDP-02 of 0.15 mg/kg to 2.0 mg/kg given at intervals of 2 weeks to 2 months longer, may be appropriate.

Follow-up blood sampling up to Study Day 212, was necessary to confirm that free α4β7 binding sites on lymphocytes had returned to baseline (pre-dose) levels. The initial reappearance of free α4β7 sites appeared to occur when LDP-02 blood concentrations became non-detectable.

Effect of LDP-02 on immune function: Lymphocyte subset analysis using flow cytometry, immunoglobulin subclass analysis, and lymphocyte stimulation with a mitogen were performed to assess effects of LDP-02 on human immune function. There appeared to be no treatment related effects on the immune function profile.

Conclusions

The administration of LDP-02 at IV doses of 0.15, 0.50, 1.50, and 2.5 mg/kg and a SC dose of 0.15 mg/kg SC to healthy male subjects was well-tolerated. There appeared to be no treatment related effects on the immune function profile. There was no detection of free $\alpha4\beta7$ binding sites on lymphocytes following administration of LDP-02 at the lowest dose (0.15 mg/kg), as measured by FACS, for approximately 2 weeks with longer periods of $\alpha4\beta7$ saturation observed at the higher doses. The non-compartmental pharmacokinetics of the lower doses of LDP-02 (0.15 and 0.5 mg/kg) were well characterized and non-linear pharmacokinetics became evident as the dose was increased up to 2.5 mg/kg. The study revealed that LDP-02 saturation of $\alpha4\beta7$ integrin on lymphocytes is remarkably and unexpectedly persistent following administration of a single dose of the antibody (Figure). At the lowest dose of LDP-02 administered once (0.15 mg/kg IV), no free $\alpha4\beta7$ binding sites (i.e., LDP-02 epitopes) could be detected for approximately two weeks after dosing. *At higher doses of LDP-02 (e.g., 2.5 mg/kg) no free $\alpha4\beta7$ binding sites could be detected for up to 70 days.*

The original study design called for measurements of pharmacokinetics and pharmacodynamics (presence or lack of $\alpha4\beta7$ binding sites) for 36 days. A 36 day measurement period was selected based on the pharmacokinetic half-life of LDP-02 in cynomolgus monkeys (approximately 4.2 days after a single administration of LDP-02 at 2.5 mg/kg) and results of studies with other antibodies. However unexpectedly, the human study demonstrated the presence of LDP-02 in serum at Study Day 36 at some doses as well as maximal saturation of $\alpha4\beta7$ on peripheral lymphocytes An amendment to the protocol was submitted to allow sampling for pharmacokinetics and pharmacodynamics to Study Day 212.

When the data from the completed study were analyzed, it was clear that there was an unexpected persistent maximal (≥85%) saturation of $\alpha4\beta7$ sites on lymphocytes after a single administration of LDP-02. The pharmacodynamic properties of LDP-02 are unexpected and could not have been predicted at the time the invention was made. This remarkably persistent saturation has a relationship to the pharmacokinetic half-life after a single administration of LDP-02 (14-17 day *in vivo* half-life of the antibody (when administered at 1.5 mg/kg or 2.5 mg/kg; Table 2 ($t_{1/2\alpha}$)). The pharmacokinetic half-life is unusual; the persistent pharmacodynamic effect of

maximal saturation up to 70 days in combination with pharmacokinetics results was unexpected and could not have been predicted.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

[signature] MD
Steven B Landau, M.D.

January 18, 2000
Date

Figure : α4β7 Saturation on CD8+ Cells